

Historic, Archive Document

Do not assume content reflects current scientific knowledge, policies, or practices.

Reserve
H351
A07

FOR OFFICIAL USE ONLY
NOT FOR PUBLICATION

United States Department of Agriculture
Agricultural Research Service

U. S. DEPT. OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY

SEP 15 1965

CURRENT SERIAL RECORDS

Report of
ANNUAL CORN AND WHEAT UTILIZATION CONFERENCE

Northern Utilization Research and Development Division
and
Corn Industries Research Foundation
Technical Committee

Peoria, Illinois

June 15, 1965

TABLE OF CONTENTS

	<u>Page</u>
SUMMARY	1
PROGRAM	3
ABSTRACTS OF TECHNICAL REPORTS AND DISCUSSIONS:	
Introduction	
R. J. Dimler, Director, Northern Division	4
Comparison of Amyloglucosidase Produced by Different Strains of <u>Aspergilli</u>	
K. L. Smiley.	6
Review of Aflatoxin Problem	
C. W. Hesseltine	7
Preparation and Composition of Glycol Glucoside Polyethers from Starch	
F. H. Otey	8
Studies on Graft Copolymers of Starch	
G. F. Fanta	9
Quantitative Analysis of Complex Mixtures of Carbohydrates by Gas Chromatography	
J. H. Sloneker	10
Hydroxyproline-Containing Mucopolysaccharide from Corn Pericarp	
Joyce A. Boundy	11
New Dispersing Methods for Preparing Undegraded Corn Starch	
S. R. Erlander	12
Progress Report on High-Amylose Corn Research	
M. J. Wolf	17
Conformation of Amylose and Amylopectin in Solution as Determined by Nuclear Magnetic Resonance	
C. A. Glass	19
DISCUSSION	20
LIST OF ATTENDANCE	21
GROUP PHOTOGRAPH	23

SUMMARY

The conference was opened with introductions by R. J. Dimler who commented briefly on personnel changes since the last meeting. Dr. Irving is now Administrator, Agricultural Research Service, and Dr. Senti, Deputy Administrator, Nutrition Consumer and Industrial Use Research. The 1964 appropriation provided funds for a new laboratory wing and extensive changes in the pilot plant, but little in new recurring funds for personnel. The current cereal research program includes in-house studies on carotenoids of corn and sorghum; nonprotein nitrogen compounds in cereals; reactions of starch and starch derivatives; cereal xanthates; and fermentation research to produce polysaccharides, xanthophylls, and spores for control of the Japanese beetle. Some starch research under contracts or grants include reactions of starch at Arizona State University; sulfur, nitrogen and phosphorus derivatives at Ohio State University; and starch-urethane foams with Archer Daniels Midland. Reports then were presented by members of the staff giving more details on specific areas of research.

Three strains of Aspergilli were studied for production of amyloglucosidase, and specific activities of products were determined. Conditions for production of aflatoxin by Aspergillus flavus were studied with maximum yields in 5 days at 25° C. and a pH of 3. Examination of 52 strains of Aspergillus oryzae used in oriental fermented foods revealed no aflatoxin production.

Research to produce starch-glycol polyethers was described. Pilot-plant scale machine production of rigid starch-urethane foams is in process and will provide information for plant process design, operating costs, and more extensive evaluation of foam properties.

Graft copolymers of starch with acrylonitrile catalyzed with ceric ammonium nitrate were prepared under various reaction conditions. The crude homopolymers were fractionated and effects of water, aqueous ethylene glycol, and aqueous dimethylformamide as reaction media were determined.

Conditions were described for preparation of trimethylsilyl ether and acetyl ester derivatives of simple sugars and their successful separation by gas chromatography. Application of the assay to the determination of anhydro sugars formed during pyrolysis of samples of starch and dextran was demonstrated.

Hydroxyproline found in corn pericarp tissue is in a polypeptide associated covalently with carbohydrate, apparently of the hemicellulose type. Properties, composition, and response to degrading enzymes were described.

The ability of various aqueous salt solutions to disperse high-amylose cornstarch (70 percent apparent amylose) was examined. Theoretical considerations for salting-in and salting-out effects were discussed, and a model was proposed to explain effects described.

Continuing progress in raising the amylose content of strains of amylomaize suitable for commercial use is indicated by the increase in number of samples analyzed in the 80-85 percent range from 1.6 percent in 1963 to 13.5 percent of samples analyzed from the 1964 crop. Differential iodine-staining reactions under crossed polars suggest that amylose content varies from cell to cell in the endosperm and even between different granules and parts of the same granule within a cell.

Proton magnetic resonance spectra of carbohydrates in deuterium oxide solution were obtained. The anomeric proton signal was easily recognized, occurring from 4.62 to 5.25 τ for equatorial protons and from 5.12 to 5.65 τ for axial protons. Data are in agreement with assignment of the C1 chair form of the pyranose ring to the linear polymers of D-glucopyranose.

In general discussion, the value to the industry of the basic research presented was emphasized.

United States Department of Agriculture
Agricultural Research Service

PROGRAM

ANNUAL CORN AND WHEAT UTILIZATION CONFERENCE

Northern Utilization Research and Development Division
and
Corn Industries Research Foundation
Technical Committee

Peoria, Illinois
June 15, 1965

Morning Session

Introduction R. J. Dimler

Comparison of Amyloglucosidase Produced by
Different Strains of Aspergilli K. L. Smiley

Review of Aflatoxin Problem C. W. Hesseltine

Preparation and Composition of Glycol
Glucoside Polyethers from Starch F. H. Otey

Studies on Graft Copolymers of Starch G. F. Fanta

Quantitative Analysis of Complex Mixtures
of Carbohydrates by Gas Chromatography J. H. Sloneker

Group Photograph

Luncheon

Afternoon Session

Hydroxyproline-Containing Mucopolysaccharide
from Corn Pericarp Joyce Boundy*

New Dispersing Methods for Preparing
Undegraded Corn Starch S. R. Erlander

Progress Report on High-Amylose
Corn Research M. J. Wolf

Conformation of Amylose and Amylopectin in Solution
as Determined by Nuclear Magnetic Resonance C. A. Glass

Open Discussion

*Presented by Dr. J. S. Wall.

INTRODUCTION

R. J. Dimler

Dr. R. J. Dimler opened the conference with the introduction of visitors and laboratory personnel. He commented briefly on administrative shifts which have placed Dr. F. R. Senti as Deputy Administrator, Nutrition, Consumer, and Industrial Use Research and Dr. G. W. Irving, Jr., as Administrator, Agricultural Research Service.

The appropriation approved by Congress in December 1963 provided for a new wing and revamping of the fermentation pilot plant. The alcohol plant, provided in the original building as part of studies of motor fuels from grain and fermentable sugars from agricultural residues, has been removed and a continuous-papermaking machine is being installed.

We have had no new funds other than nonrecurring funds from CCC so have had no staff increases other than a small increase for studies of mycotoxins. We have used these nonrecurring funds, therefore, for research equipment and for contracts and grants. Our research program covers cereals, oilseeds, and new industrial crops but only our cereals research program will be discussed today. Because of limitations on time, only brief mention is possible for a number of subjects; detailed reports on selected ones have been included in the program.

Basic studies on carotenoids of corn and sorghum have revealed some corn samples with up to 77 p.p.m. xanthophylls compared to levels of about 25-30 p.p.m. for the usual dent corn. Problems of stability during storage and during processing by wet- and dry-milling methods need study. We also are looking at nonprotein nitrogen compounds. Other studies include properties and reactions of starch and use of NMR to determine conformation of amylose and other carbohydrates. Contract research at the University of Arizona on reactions of starch and glucosides with acetylene and mercaptans has indicated that the maximum degree of substitution averaged one acetylene per anhydroglucose unit, but the presence of disubstituted units showed that the reaction was not homogeneous. At the Northern Division, Mr. Hodge is studying reactions of maltose and dextrose. Contract research at Ohio State University includes sulfur, nitrogen, and phosphorus derivatives of starch.

Disulfide bonding in wheat proteins is being studied in relation to structure and properties. Corn protein research has not been resumed since Dr. Turner left. Contract research on wheat and corn at Purdue includes studies on proteins, industrial products from starch and flour and the nature and structure of high lysine corn. Studies on graft copolymers with starch which were started at Stanford Research Institute are now being continued at the Northern Division. Part of our research on starch-urethane foams is being done under contract with Archer Daniels Midland. Studies on xanthates are continuing at Peoria on the chemical structure and time-dependent rearrangements and on ex situ crosslinking to extend the utility of these products.

Fermentation research involves continuing screening of our ARS Culture Collection, a world source of industrial microorganisms for strains having specific uses. Interest continues in polysaccharides; B-1459 is in commercial production (Kelco, with three other firms on a pilot-plant scale) and appears on the verge of FDA approval for use in food. Continuous fermentation techniques are being worked out on B-1459 and such methods would have commercial application for other high-viscosity fermentations where oxygen supply and agitation are problems. Studies on β -carotene production have been extended to xanthophylls, but practical yields have not yet been attained. Studies on the Japanese Beetle, directed toward commercial production of milky disease spores, have become a long-term fundamental study on sporulation mechanisms. We can now produce spores on solid media and sporelike forms in liquid media. Research here and under grants to several universities have revealed a specific factor controlling sporulation.

COMPARISON OF AMYLOGLucOSIDASE PRODUCED BY DIFFERENT
STRAINS OF ASPERGILLI

K. L. Smiley

A comparison was made of production and properties of amyloglucosidase elaborated by Aspergillus niger NRRL 337, A. awamori NRRL 3112, and an unidentified culture, NRRL 3122, belonging to the A. niger group. Crude extracts of the three cultures were subjected to chromatography on DEAE cellulose. The eluted enzymes were compared with one another for specific activity and amount of transglucosylase present. All the cultures showed two or more peaks exhibiting typical amyloglucosidase activity. The specific activities of the different amyloglucosidase fractions from a single culture showed differences, but the glucose-producing capabilities and the lack of transglucosylase were about the same for the different fractions. In addition to the amyloglucosidase fractions, A. niger showed a pronounced peak of transglucosylase activity as evidenced by formation of isomaltose from maltose. The results of the study provide a method for selection of strains with little or no transglucosylase activity.

Discussion. A question was raised as to the time of fermentation. Dr. Smiley indicated that 4 days at 60° F. is near optimum. Another question was the attitude of Food and Drug toward use of A. awamori for glucose conversion. This has not been checked.

REVIEW OF AFLATOXIN PROBLEM

C. W. Hesseltine

Aflatoxin can be produced by Aspergillus flavus either on solid or liquid media (still or shaken) made from potato-dextrose. Aflatoxin occurs in culture filtrates, mycelium, and spores. Examination indicates that certain strains, e.g., NRRL 482, do not produce aflatoxin under the conditions used, whereas, others vary greatly in the amounts formed. Aflatoxin is produced as early as 48 hours, but maximum yields are usually obtained in fermentation liquors in 5 days. Often amounts in culture liquors decrease rapidly after 5 days. Associated with toxin formation is a drop in pH to 3 when some of the best producers, such as NRRL 2999 and NRRL 3000, are used. Examination of 52 strains of Aspergillus oryzae obtained from pure culture starters, used in Japan to manufacture shoyu, miso, and black beans, showed 37 that produced fluorescent material, but there was no evidence that the fluorescing substances were aflatoxin. A number of good food samples including miso, shoyu, and various types of tempeh were examined, and although they had a great deal of fluorescent material, none contained aflatoxin at levels detectable by the methods used.

Discussion. Several questions were raised clarifying points discussed. Data presented on corn are part of the stratified grade survey, a random sampling of commercial grades of grain. There is no indication that the low pH of wet-milling will destroy aflatoxin. Both Massachusetts Institute of Technology and the Northern Division show that a low pH is necessary to get aflatoxin. Above pH 4 aflatoxin is not produced. Substances such as ethylene oxide, propylene oxide, and hydrogen peroxide will kill the organism, but not necessarily destroy the aflatoxin. There are cases, however, where the organism itself is a pathogen, and can grow in the body. Temperature affects aflatoxin production: At 37° C. no aflatoxin is produced; aflatoxin is produced over the range 20-28° C. with the optimum around 25° C.; oxygen is required.

In answer to a question on the status of knowledge of the toxicity in humans, it was brought out that no experimental data are available on response of humans to aflatoxin. However, 30 p.p.b. is considered toxic and carcinogenic for some species. For rats and perhaps other animals, there is some indication that 4.5 p.p.b. may also be carcinogenic. Action of the toxin includes "sticky chromosomes." Research in England with monkeys has indicated, surprisingly, some individuals are immune, some susceptible. It was stated that a government specification has been received calling for, "not more than 1 p.p.b. aflatoxin." Methods used by Dr. Shotwell and Food and Drug detect as low as 3 p.p.b. and are semiquantitative, with 3-6 p.p.b. a practical detectable range. The chick embryo assay is reported accurate to less than 10 p.p.b.

PREPARATION AND COMPOSITION OF GLYCOL GLUCOSIDE POLYETHERS FROM STARCH

F. H. Otey

The acid-catalyzed reaction of starch with ethylene glycol at 120-130° C. (I+EC Product Research and Development 2: 256-259 (1963)) yields a mixture of isomeric glycosides that has potential for use as an industrial polyol. Separation of the major glycosides present was accomplished by carbon-Celite column adsorption followed by gradient elution. Quantitative gas-liquid chromatographic analysis showed that the crude glycoside mixture contained 45-48 percent glycol α -D-glucoside, 21-24 percent β -anomer, and at least 11 percent glycol diglucoside.

The influence of amount of acid catalyst and reaction time during trans-glycosylation and also of propylene oxide concentration on viscosity and hydroxyl number of polyethers was determined. Over the range studied, acid catalyst concentration and reaction time did not significantly affect these properties. The application of select conditions gave viscosity and hydroxyl number reproducibility that were well within the limits of commercial acceptability.

Pilot-plant studies on process development and cost analysis of glycol glycosides and glycol glycoside polyethers for the production of rigid urethane foams are nearing completion. Pilot-plant-scale machine production of foams is scheduled for next month and will complete the experimental studies. Final process design, plant and operating costs, and evaluation of foam properties will form the subject of a publication to complete the study.

Discussion

The question was raised as to when the pilot-plant studies by ADM will be available. Runs will be made of 2,000 pounds of polyethers, so samples should be available this fall or a little later. To a question about the use of sucrose, it was indicated that sucrose has a disadvantage of high viscosity as well as a price disadvantage compared to starch.

STUDIES ON GRAFT COPOLYMERS OF STARCH

G. F. Fanta

The ceric ammonium nitrate catalyzed graft copolymerization of gelatinized wheat starch with acrylonitrile was studied under a number of reaction conditions, and the crude copolymer fractionated according to its solubility in dimethylformamide, γ -butyrolactone, and dimethylsulfoxide. Amount of homopolymer, average molecular weight of grafted polyacrylonitrile chains, and grafting frequency (average number of AGU per grafted chain) were determined. In comparison with water as a reaction medium, aqueous dimethylformamide acted to increase the amount of homopolymer, decrease the overall amount of acrylonitrile grafted, and increase the average number of AGU per grafted chain. Aqueous ethylene glycol (50 percent) led to almost exclusive formation of homopolymer. Although water was the preferred reaction medium, the graft copolymer was still found to be infrequently grafted (Mn of grafted chain: 840,000; grafting frequency: 4600 AGU/graft). Neither the method of addition of catalyst and monomer (addition of monomer either before or after catalyst) nor an increase in catalyst concentration was found to alter the results to any great extent. A reaction temperature of 50° as opposed to 25° caused little variation in copolymer composition; however, at 2° an appreciable amount of very heavily grafted product with somewhat shorter grafted chains was formed. In a preliminary study on the effect of chain terminators, neither chloroform nor oxygen was found to affect materially the molecular weights of grafted chains.

Discussion

Questions raised were answered as follows:

Molar ratios used were 0.6 mole acrylonitrile, 0.135 mole starch.

Properties of the grafted product of note: infusible, swelled greatly in DMSO but did not dissolve. The starch molecule appears still intact; a graft replaces an H atom, but which one is not known. The polymer is a white powder. Polyacrylonitrile side chains are long with a molecular weight of 7-8,000.

QUANTITATIVE ANALYSIS OF COMPLEX MIXTURES OF CARBOHYDRATES
BY GAS CHROMATOGRAPHY

J. H. Sloneker

Separation of free sugars as their trimethylsilyl ether or acetyl ester derivatives is complicated by the formation of 2 to 4 isomers during derivatization. However, the liquid phase, Carbowax 20M (15 percent on 80-100 mesh Chromosorb W, 1/4 in. x 12 ft.) was shown to be an excellent phase for the quantitative separation of the trimethylsilyl ether derivatives of D-mannose, D-galactose, and D-glucose.

Complex mixtures of monosaccharides could be quantitated with ± 2 percent accuracy if they were first converted to their corresponding alditols by reduction with sodium borohydride. The alditol acetates were readily separated on an ethylene glycol succinate-cyanoethyl silicone copolymer, ECNSS-M (3 percent on 100-120 mesh Gas Chrom Q, 1/4 in. x 10 ft.).

Quantitative separation of the anhydrosugars--1,6 anhydropyranoses and 1,6 anhydrofuranoses of D-galactose and D-glucose was achieved on the cyanoethyl silicone polymer, XE-60 (5 percent on 80-100 mesh Chromosorb W, 1/4 in. x 8 ft.). Application of the assay to the determination of anhydro sugars formed during pyrolysis of samples of starch and dextran was reported.

Discussion

Several questions were raised concerning application and limitations of the method; for example, could you analyze a jar of jam? Sugars must be in a state to be reduced and acetylated, and be free from interfering substances. Tetrasaccharides are about the present limit of molecular size. The short carbowax column is limited to 250° C. It is still good after 6 months' use.

HYDROXYPROLINE-CONTAINING MUCOPOLYSACCHARIDE
FROM CORN PERICARP

Joyce A. Boundy

Hydroxyproline found in corn pericarp tissue occurs in a polypeptide associated covalently with hemicellulose. The hydroxyproline-containing substance was extracted from pericarp by an alkaline copper-sulfite reagent. However, it was soluble in and extracted by 15 percent trichloroacetic acid, which precipitates protein impurities devoid of hydroxyproline. Such treated material appears homogeneous upon chromatography on cross-linked dextran and on carboxymethyl cellulose columns. A preparation containing 2 percent N was shown by amino acid analysis to have 0.8 g. hydroxyproline per gram N, along with other amino acids. The ratio of carbohydrate to nitrogen varied with extraction conditions, indicating that extraction involved some degradation. The material was partially digested by a hemicellulase but was not readily attacked by the enzymes pronase or papain. Xylose, glucose, galactose, and arabinose were the major sugar components. These findings are consistent with those of Lamport (Federation Proc. 21, 398 (1962)) who proposes a structural role for similar materials in plant cell walls.

Discussion

Points brought out in discussion include that there were no hydroxyproline-containing mucopolysaccharides in the endosperm. PL-480 studies on mucopolysaccharides of wheat have indicated that in wheat these are mostly pentosans along with albumins without hydroxyproline.

NEW DISPERSING METHODS FOR PREPARING UNDEGRADED CORN STARCH

S. R. Erlander

A model is proposed that explains the resulting values for the dielectric decrement and dielectric constant and the salting-in and salting-out behavior of different ions. According to the proposed model, for the ions H^+ , Li^+ , Na^+ , F^- , SO_4^{2-} , and possibly OH^- , there exists a monomolecular hydration shell of tightly bound water molecules which are positively hydrated. (For definition of positive and negative hydration and A- and B- shells, see references 1 and 3.) For Li^+ and H^+ ions there exists a second or outer monomolecular hydration shell which is more loosely attached or negatively hydrated. Because of the manner in which water associates with anions, the positively hydrated anions cannot produce a second negatively hydrated shell. The ions, K^+ , Rb^+ , Cs^+ , Cl^- , Br^- , I^- , SCN^- , and similar ions possess a monomolecular shell of negatively hydrated water molecules. The model and the calculated effective dielectric constant are used to explain such phenomena as hydrophobic bonding, random coil-helix transitions in nucleic acids and proteins, and cell-wall permeability to glucose and cations. The ability of an ion or dipolar molecule to disperse hydrogen or hydrophobic bonds is a direct function of the charge per unit surface area of the ion or dipolar molecule which is manifested as its effective dielectric decrement.

The ability of various aqueous salt solutions to disperse high-amylose corn starch (70 percent apparent amylose) was examined. The results were interpreted in light of the above proposed theory concerning the structure and properties of hydrated ions and molecules. In those salts where both cation and anion have negative hydration (possess only B-regions) such as guanidinium chloride or guanidinium thiocyanate, the solubility of starch continued to increase to the saturation point of the salt. On the other hand, if the cation has positive while the anion has negative hydration, then the solubility of starch goes through a maximum (at 6 M LiBr, 6 M LiSCN or 9 M LiCl). If a salt having only negatively hydrated domains is added to the maximum dispersing solvents, 6 M LiBr or 6 M LiSCN, then an increase rather than a decrease in dispersion power is obtained because the added salt increases the volume of the negatively hydrated domains. The maximum in the dispersion curve for LiBr or LiSCN is obtained because of a reduction or elimination of negatively hydrated water. The positively hydrated water surrounding the Li^+ ion is not readily exchangeable with the medium and therefore the starch molecules cannot exist in these domains. The primary function of the hydrated lithium ion is to increase the effectiveness of the negatively hydrated domains of its anion or any added salt by eliminating their outlying areas and by maintaining the less hydrated anion (or added salts) in solution. If both anion and cation have positively hydrated domains such as in $MgSO_4$, then a poor dispersing solvent is obtained. At high temperatures some of the positively hydrated water will be converted to negatively hydrated water and hence the starch will be made soluble.

In order to understand the solvent system more fully, let us calculate the volume of the hydrated ions. The volume in ml. that 1 mole of a hydrated ion will occupy is $V = 6.023 \times 10^{23} (4/3) \pi R^3 = 2.523r^3$ where r is in Angstrom units. Substituting the hydrated radii given by Nightengale (2) into this expression gives $V = 141$ ml. for 1 mole of hydrated Li^+ ion and $V = 91$ ml. for 1 mole of hydrated Br^- ion or a total volume of 232 ml. for 1 mole of LiBr . Thus at 25°C ., 4.3 moles of hydrated LiBr occupy a volume of 1 liter.

Such calculations can be extended to higher concentrations of salt. The lithium ion is both positively and negatively hydrated in dilute solutions, i.e., contains both A- and B- regions according to the Frank and Wen (1) nomenclature. On the other hand, the chloride, bromide, and thiocyanate ions are only negatively hydrated. The structure of the Li^+ , Cl^- , and Br^- ions can be represented as regions whose distance from one shell to the next represent so many Angstrom units as shown in Table I. Thus the hydrated radius, r_H , of the Li^+ ion is a sum of the crystal radius, r_x , plus the diameter of a water molecule for the A-region and plus an average distance for the B-region which depends on how many water molecules are attached or hydrated in the B-region. The latter number is a function of the charge per unit surface area. The lower the value of this factor, the less will be the ability of the ion to hold water molecules. In other words, the lower the charge per unit surface area and, consequently, the lower the radius for the B-region, the faster will be the exchange of an average hydrated water molecule with the medium and the shorter will be its hydration time. Therefore, the B-region of the chloride ion holds a water molecule for a longer period of time than that of the bromide ion or that of the lithium ion.

Using this model we can calculate the average structure of the hydrated ions at a particular molarity. As shown above, about 4.3 moles of the hydrated LiBr occupy a volume of 1 liter. As solid LiBr is added to the 4.3 M LiBr solution, the water of hydration in the B-region of the lithium ion will disappear first followed by that in the B-region of the bromide ion because of the preferential hydration (greater exchange time) of the different regions. For LiCl and LiSCN the same sequence will occur.

In Table II the molarities at which these average structures exist have been tabulated. The LiBr is not able to disperse starch granules until its hydrated ions can theoretically occupy or engulf the entire medium. That is, all of the hydrogen bonds of a single starch chain must be broken at the same time before that chain (or a segment of it) can be released or dispersed. This situation can only happen when the volume of the hydrated ion approaches the volume of the solution.

The dispersing power of the LiBr increases with an increase in its molarity because now the B-regions are being reduced to make them more effective. In other words, the ions in solution undergo Brownian movement which causes

sporadic increases or decreases in the concentration of LiBr. As the concentration of LiBr is increased, the occurrence of sporadic C-regions is diminished and the probability that a water molecule will remain in the B-region of a bromide ion is increased. As shown in Table II, at 8.7 M LiBr, the average bromide ion will not be hydrated, i.e., all B-regions will have disappeared. Since the favorable dielectric properties for dispersing starch granules will depend on the existence of this B-region, then the maximum in dispersing power at about 6.0 M LiBr is understandable. The increase in dispersing power with an increase in molarity is due to an increase in the effectiveness of the B-regions of the bromide ions, while the corresponding decrease after 6.0 M LiBr is due to the disappearance of the B-regions.

The same reasoning can be applied to LiSCN, which also has a maximum dispersion power at 6.0 M solution. The dispersibility at lower molarities and the broader maximum of the LiSCN solutions as compared to the LiBr solutions must be due to the larger radius of hydration of the thiocyanate ion. The LiCl does not disperse starch at 4 to 6 molar concentrations because the hydrated chloride ion produces an unfavorable dielectric constant. The effective dielectric constant of the chloride ion is about $D = 45$ which is much less than that of water ($D = 78.3$). The bromide and thiocyanate ions have effective dielectric constants greater than that of water.

As shown in Table II, LiCl solutions do not begin to disperse starch granules until the B-region of the Cl^- ion has started to disappear. In other words, the dispersion power of LiCl begins at a molarity which gives the maximum dispersion power for the LiBr or LiSCN. The maximum dispersion power for the LiCl occurs when the average chloride ion is unhydrated. The ability of the unhydrated chloride ion to disperse starch granules and the inability of the unhydrated bromide or thiocyanate ions to do so is a result of the greater length of time that a water molecule or the hydroxyl group on starch molecules can remain hydrated to the chloride ion. In order for an amylose or amylopectin chain to become released from adjacent chains to which it is hydrogen bonded, all of the hydrogen bonds of the entire chain or section of chain must be destroyed at a specific time; but the bromide and thiocyanate ions would release their hydrated hydroxyl groups at a much faster rate than the chloride ion. Consequently, the length of time that a chloride ion holds onto a hydroxyl group must be sufficient to allow the simultaneous formation of such chloride hydrates all along the glucosidic chain. The result is a release of the chain from the adjacent chains to which it is hydrogen bonded. Further increases in the concentration of LiCl past the maximum dispersing value of 9 M LiCl results in a decrease in the concentration of chloride ion. In other words, with the loss of water molecules in its A-region the lithium ion is now able to form a salt bond with the chloride ion. The result is a decrease in the availability of the chloride ion and a consequent decrease in the dispersing power of the LiCl solution.

The dispersion ability of guanidinium chloride and guanidinium thiocyanate can be explained in a similar manner even though r_x and r_H are not known

for the G^+ ion. The chloride ion cannot disperse starch unless it becomes unhydrated. Consequently, the dispersing properties of $G\text{HCl}$ are due entirely to the guanidinium ion because the Cl^- ion cannot become unhydrated if it has a counter-ion such as G^+ . In the case of $G\text{SCN}$, both the anion and cation are able to disperse starch granules, and hence, the $G\text{SCN}$ has greater dispersing ability than $G\text{HCl}$. In addition, the fact that the hydrated SCN^- ion can disperse starch granules while the hydrated Cl^- ion cannot accounts for the dispersion power of $G\text{SCN}$ at a lower molarity than that of $G\text{HCl}$. The $G\text{HCl}$ begins to disperse starch at a concentration which is greater than the corresponding concentration of LiBr because the A-region of the lithium ion has effectively increased the concentration of the bromide ion.

The solubility of benzene, benzoic acid, and hexanol in aqueous solutions containing various salts or dipolar molecules was also examined. For many salt solutions, the solubility of benzene was found to be a linear function of the molar concentration of salt relative to the saturation point of the salt. This result agrees with the proposal that ions other than Li^+ and H^+ ions have only a single monomolecular layer of hydration. The association of NH_4^+ and OH^- ions to form the dipolar NH_4OH molecule reduces the amount of electrostatic charge per surface area and, hence, the solubility of benzene in NH_4OH solutions is increased. In concentrated solutions of NH_4Cl or HCl , there appears to be electrostatic interactions between the NH_4^+ and Cl^- and the H^+ and Cl^- ions which reduce the charge per unit surface area on these ions. Examination of the ability of various ions to salt-in suggests that the guanidinium ion has maximum salting-in properties. Larger cations which salt-in benzene to greater extents such as the tetraethylammonium ion do so by forming hydrophobic bonds rather than by having a better value of charge per unit area. Thiourea salts-in more than urea because of less charge per unit surface area. These results also suggest that urea exists almost as a zwitterion. Sulfate and carboxylate ions salt-out more than nitrate ions because of the absence of a positive electrostatic charge on the carboxylate ion and because of tetrahedral structure of the sulfate ion. Thus, unlike dimethyl sulfoxide, the positive charge on the sulfate ion is cancelled by the engulfing oxygen atoms. Positive hydration is shown to exist for anions. These results are applied to the properties of proteins.

References

- (1) Frank, H. S., and Wen, W. Y., Discussions Faraday Soc. 24, 133 (1957).
- (2) Nightengale, E. R., Jr., J. Phys. Chem. 63, 1381 (1959).
- (3) Samoilov, O. Ya., Discussions Faraday Soc. 24, 171 (1957).

Table I. Structure of Hydrated and Unhydrated Li^+ , Cl^- , or Br^- Ions*

Ion	Thickness of Shells			
	r_x	A-region	B-region	r_H
Li^+	0.60	+ 2.76	+ 0.46	= 3.82 Å
Cl^-	1.81	+ 0	+ 1.51	= 3.32 Å
Br^-	1.95	+ 0	+ 1.35	= 3.30 Å

* Crystal radii, r_x , are those of Pauling.

Table II. Relationship Between Dispersion of Starch Granules and Structure of Ions*

Structure of Li^+	$\text{Li}^++\text{A}+\text{B}$	Li^++A	Li^++A	Li^+	<u>Dispersion</u>	
Structure of Br^-	Br^-+B	Br^-+B	Br^-	Br^-	Initiation	Maximum
Moles /l. of LiBr	4.3	5.4	8.7	52	4.0	5.5-6.5
Structure of Cl^-	Cl^-+B	Cl^-+B	Cl^-	Cl^-		
Moles /l. of LiCl	4.3	5.3	9.0	64	5.8	8.5-9.0

* Data for calculating the number of moles required for the different models of LiBr or LiCl to occupy 1 liter were obtained from Table I.

PROGRESS REPORT ON HIGH-AMYLOSE CORN RESEARCH

M. J. Wolf

There was further progress in raising the amylose content of amylomaize during the past year (ending January 1965). The highest amylose content found so far in corn breeders' selections was 85 percent apparent amylose; the percentage of samples analyzing between 80 and 85 percent apparent amylose rose to 13.5 percent of the total samples submitted, compared with only 1.6 percent the previous year (1963). Considerable progress has been made by cooperating corn breeders toward producing commercial hybrid stock containing 80-85 percent amylose. Since 1959 this so-called "elite" stock has been registering an average advance of 2-3 percent amylose per year; it is anticipated that commercial grade amylomaize in the 80 to 90 percent amylose range should be available within the next few years. The highest commercial grade available presently is amylomaize with starch in the 70-80 percent range.

Amylose distribution in corn kernels at various positions on the ear and at different levels within the kernels is important theoretically and also for sampling for amylose analysis. Distribution studies showed a gradient of increasing amylose in kernels from the tip of the ear to the butt end; differences from tip to butt were as high as 11 percentage points. In kernels, the highest amylose content was found in the mid-section and the amount decreased at the crown and tip cap. The variation in kernels was from 0 to 3 amylose percentage points.

Starches stained with iodine showed a different starch-iodine coloration when examined between crossed polars; ordinary corn starch granules with about 27 percent amylose are bright blue, high-amylose starch granules (70 percent plus) are red. In corn at intermediate amylose levels (35 to 65 percent) a mixture of starch granule types is found including blue granules, red granules, and variegated granules showing both red and blue coloration. This suggests that in corn at intermediate amylose levels the amylose content of starches varies in different cells of the endosperm, in different starch granules in the same cell, and even in different portions of a given starch granule.

Polarization colors of starch stained with iodine may be useful in screening corn selections for high amylose. Corn with starch showing only red polarization colors appears to correspond to corn above 65 to 70 percent amylose. Additional study is needed to determine the significance of the polarization colors in terms of amylose contents. A red polarization color in a starch is correlated with a V-type x-ray pattern; a V-type x-ray pattern indicates presence of helical glucose chains in the starch.

Discussion

Several questions were raised for which we do not have adequate information. Variation in distribution of amylose in regard to position of kernel on the ear (base to tip) and within individual kernels (base to crown) indicates

a need for more information on this and on variation in other constituents, especially oil and protein. Effect of maturity on amylose content also needs study. Starch-iodine staining reactions under crossed polars present a different picture than when ordinary surface lighting is used, although the light source is an ordinary tungsten filament microscope light. The concentration of iodine used was around 0.3 percent, but the stain is commonly introduced from one side of the cover slip so there is a concentration gradient across the slide as the initial staining reactions are observed. High light intensity is used with the crossed polars.

CONFORMATION OF AMYLOSE AND AMYLOPECTIN IN SOLUTION
AS DETERMINED BY NUCLEAR MAGNETIC RESONANCE

C. A. Glass

The proton magnetic resonance spectra of carbohydrates in deuterium oxide solution were obtained and considered with respect to probable conformation. The anomeric proton signal occurred at lowest field and was easily recognized. In the various glucose derivatives investigated, this signal occurred from 4.62 to 5.25 τ for equatorial protons and from 5.12 to 5.65 τ for axial protons. Within each of these ranges, the chemical shifts varied inversely with the electronegativity at the C₁ site, as calculated by the technique of Gordy (1). Variable electronegativity at C₁ accounts for the overlap of ranges for equatorial and axial protons, thereby bringing the NMR data into agreement with assignment of the C1 chair form of the pyranose ring to the linear polymers of D-glucopyranose. The orientation of the glucopyranose rings in cyclodextrins is shown to preclude ring current effects, and the up-field chemical shifts of these materials are shown to be consistent with the C1 form.

Reference

- (1) Gordy, W. A Relation Between Bond Force Constants, Bond Orders, Bond Lengths, and the Electronegativities of the Bonded Atoms. J. Chem. Phys. 14 (5): 305-320 (1946).

DISCUSSION

In general discussion, a few additional points were clarified and some comments made. Hydroxyproline-containing mucopolysaccharides have not been found in corn germ, only in the structural parts--pericarp, husks, cell walls. We have not studied germ and endosperm as extensively as pericarp, however, since previous amino acid analyses had indicated that most of the hydroxyproline of the kernel was concentrated in the pericarp. Tissues are more organized than we suspected. We know little about the composition of membranes and lipid components. Plant research has lagged behind animal and microbial studies in this respect. We have wanted more basic research--this is it. Future plans should include more research on gas chromatography of carbohydrates and other basic research. Basic research opens our eyes to many possibilities and is the key to future progress. Representatives present from industry and from the Corn Industries Research Foundation can help get this message to Congress in support of our basic research.

United States Department of Agriculture
Agricultural Research Service

ANNUAL CORN AND WHEAT UTILIZATION CONFERENCE

Northern Utilization Research and Development Division
and
Corn Industries Research Foundation
Technical Committee

Peoria, Illinois
June 15, 1965

List of Attendance

CORN INDUSTRIES RESEARCH FOUNDATION TECHNICAL COMMITTEE

Corn Industries Research Foundation, Inc., Washington, D.C.

W. J. Hoover, Administrative Vice President

American Maize-Products Company, Roby, Indiana

E. L. Powell, Associate Director of Research
C. H. Hullinger

Anheuser-Busch, St. Louis, Missouri

B. L. Scallet, Associate Director, Central Research

Clinton Corn Processing Company, Clinton, Iowa

G. T. Peckham, Jr., Research Director
R. V. MacAllister, Assistant Research Director
J. M. Newton, Technical Assistant to Vice President, Sales

Corn Products Company, Argo, Illinois

Harry Gehman, Director of Research
A. L. Wilson, Assistant Director of Research
W. E. Brinker

Hercules Powder Company, Wilmington, Delaware

R. E. Chaddock, Director of Development

The Hubinger Company, Keokuk, Iowa

J. M. Seitz, Director of Research
Roger Yarbrough, Chief Chemist
L. H. Elizer

National Starch and Chemical Corporation, Plainfield, New Jersey

M. W. Rutenberg, Section Leader, Starch Research

Penick and Ford, Ltd., Cedar Rapids, Iowa

E. T. Hjermstad, Director of Research
W. C. Black

A. E. Staley Company, Decatur, Illinois

E. E. Fisher, Director of Chemical Research

NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

R. J. Dimler, Director
W. C. Witham, Assistant Director
D. L. Miller, Assistant Director
L. L. McKinney, Assistant Director
J. E. Hubbard, Assistant to Director
W. C. Schaefer, Assistant to Director

Cereal Products Laboratory

C. E. Rist, Chief	H. E. Smith
C. R. Russell	T. R. Naffziger
G. F. Fanta	C. L. Mehltretter
L. A. Gugliemelli	A. M. Mark
Florence L. Bennett	F. H. Otey
	W. B. Roth

Cereal Properties Laboratory

J. S. Wall, Acting Chief	J. S. Sawardeker
M. J. Wolf	G. E. Babcock
H. F. Zobel	J. P. McGuire
C. A. Glass	Ruta M. Purvinas
S. R. Erlander	A. D. French
J. H. Sloneker	J. Lehrfeld
R. Tobin	F. R. Dintzis

Engineering and Development Laboratory

E. L. Griffin, Chief	O. L. Brekke
R. A. Anderson	A. C. Stringfellow

Fermentation Laboratory

C. W. Hesseltine
K. L. Smiley